

What is claimed is:

1. A process for obtaining osteogenic proteins from mammalian bone tissue comprising:

contacting bone tissue with an acidic demineralization medium to provide demineralized bone tissue and a mineralized supernatant solution;

separating the mineralized supernatant solution from the demineralized bone tissue;

removing at least part of the mineral component of the mineralized supernatant solution [by contacting the mineralized supernatant solution with a mineral precipitation agent] to provide a protein supernatant solution;

extracting osteogenic proteins from the protein supernatant solution by contacting the protein supernatant solution with a protein extraction agent to provide an extracted protein medium; and

recovering osteogenic proteins from the extracted protein medium.

2. The method of claim 1 wherein said recovering step comprises

filtering said extracted protein medium in a first ultrafiltration step using a first ultrafiltration membrane having a nominal molecular weight cutoff corresponding to a high molecular weight limit to provide a permeate comprising a first osteogenic solution;

filtering the first osteogenic solution in a second ultrafiltration step using a second ultrafiltration membrane having a nominal molecular weight cutoff corresponding to a low molecular weight limit to provide a retentate comprising a second osteogenic solution; and

purifying the osteogenic proteins in said second osteogenic solution.

3. The method of claim 2 wherein said protein extraction agent comprises guanidine hydrochloride.

5 4. The method of claim 3 wherein said purifying step comprises removing said guanidine hydrochloride by at least one diafiltration step in which the osteogenic proteins are diafiltered into a diafiltration medium that does not comprise guanidine hydrochloride.

10 5. The method of claim 4 wherein said purifying step further comprises at least one purification operation selected from the group consisting of lyophilization and precipitation.

6. The method of claim 3 wherein said purifying step comprises a first diafiltration step in which at least a portion of the guanidine hydrochloride is removed by diafiltering the osteogenic protein into a first diafiltration medium comprising urea, and

15

a second diafiltration step in which at least a portion of the urea is removed by diafiltering the osteogenic protein into a second diafiltration medium comprising dilute hydrochloric acid.

20

7. The method of claim 6 wherein said purifying step further comprises lyophilizing the proteins from the second diafiltration medium to provide a solid osteogenic protein mixture.

8. The method of claim 7 wherein said purifying step further comprises  
dissolving said solid osteogenic protein mixture in a first purification medium  
comprising dilute hydrochloric acid;  
precipitating the proteins by contacting the first purification medium with a  
5 protein precipitating agent;  
separating the precipitated proteins from the first purification medium and the  
protein precipitating agent; and  
dissolving the separated and precipitated proteins in a second purification medium  
comprising dilute hydrochloric acid; and  
10 lyophilizing the proteins from the second purification medium to provide solid  
osteogenic proteins.

9. A method for isolating osteogenic proteins from mammalian bone tissue  
comprising:

15 demineralizing bone tissue in an acid medium to provide demineralized bone  
tissue and a mineral-containing acid supernatant;  
removing at least a portion of the minerals from the mineral-containing acid  
supernatant to provide a protein supernatant solution;  
extracting osteogenic proteins from the protein supernatant solution with a protein  
20 extraction agent to provide an extracted protein medium; and  
recovering osteogenic proteins from the extracted protein medium.

10. The method of claim 9 wherein the acid medium comprises hydrochloric acid.

11. The method of claim 9 wherein said removing step comprises contacting the mineralized supernatant solution with a mineral precipitation agent.

12. The method of claim 11 wherein the mineral precipitation agent comprises  
5 calcium oxalate.

13. The method of claim 9 wherein said extracting step comprises contacting said protein supernatant solution with guanidine hydrochloride.

10 14. The method of claim 9 wherein said recovering step comprises  
filtering said extracted protein medium in a first ultrafiltration step to remove  
proteins having a molecular weight exceeding a desired high molecular weight limit to  
provide a first filtered solution;  
filtering the first filtered solution in a second ultrafiltration step to remove  
15 proteins having a molecular weight below a desired low molecular weight limit to  
provide a second filtered solution; and  
purifying the osteogenic proteins in said second filtered solution.

15 15. The method of claim 14 wherein said purifying step comprises removing said  
20 protein extraction agent by at least one diafiltration step in which the osteogenic proteins are  
transferred to a medium that does not comprise the protein extraction agent.

16. The method of claim 15 wherein said protein extraction agent comprises  
guanidine hydrochloride.

17. The method of claim 15 wherein said protein extraction agent comprises urea.

18. The method of claim 15 wherein said purifying step comprises a first diafiltration  
5 step in which the osteogenic proteins are transferred to a medium that does not comprise the  
protein extraction agent, and a second diafiltration step in which the osteogenic proteins are  
transferred to a dilute acid medium that does not comprise the protein extraction agent.

19. The method of claim 15 wherein said purifying step further comprises at least one  
10 purification operation selected from the group consisting of lyophilization and precipitation.

20. The method of claim 14 wherein said protein extraction agent comprises  
guanidine hydrochloride and said purifying step comprises

a first diafiltration step in which the guanidine hydrochloride is removed by  
15 diafiltering the osteogenic protein into a first diafiltration medium comprising urea, and

a second diafiltration step in which the urea is removed by diafiltering the  
osteogenic protein into a second diafiltration medium comprising dilute hydrochloric  
acid.

21. The method of claim 20 wherein said purifying step further comprises  
20 lyophilizing the proteins from the second diafiltration medium to provide solid  
osteogenic proteins.

22. The method of claim 21 wherein said purifying step further comprises

dissolving said solid osteogenic proteins in a first purification medium comprising dilute hydrochloric acid;

precipitating the proteins by contacting the first purification medium with a protein precipitating agent;

5 separating the precipitated proteins from the first purification medium and the protein precipitating agent; and

dissolving the separated and precipitated proteins in a second purification medium comprising dilute hydrochloric acid; and

10 lyophilizing the proteins from the second purification medium to provide purified osteogenic proteins.

23. The method of claim 22 wherein said protein precipitating agent comprises acetone.

15 24. A method for isolating osteogenic proteins from mammalian bone tissue comprising:

demineralizing bone tissue in an acid medium to provide demineralized bone tissue and a mineral-containing acid supernatant;

20 extracting osteogenic proteins from the protein supernatant solution with a protein extraction agent to provide an extracted protein medium; and

recovering osteogenic proteins from the extracted protein medium.